HCV infection is common and associated with significant morbidity and mortality among dialysis patients and is more common in dialysis patients than in healthy populations. Dialysis Outcomes and Practice Patterns Study, which provides reliable data regarding the prevalence of HCV infection among dialysis patients, is a prospective, observational survey among adult hemodialysis patients who are randomly selected from 308 representative dialysis facilities in many countries such as Japan, France, Germany, Spain, Italy, the United Kingdom, and the United States. In the 2004 report, the overall prevalence was 13.5% (compared to global prevalence in the general population indicating greater risk of getting HCV infection among patients undergoing hemodialysis). Among hemodialysis patients; the number of blood transfusions, duration of the hemodialysis treatment, and also nosocomial transmissions due to poor infection-control measures are among the most important ones.

HCV is a RNA virus, which belongs to the family Flaviviridae. The HCV virion measures 55-65 nm in diameter. The HCV virion RNA contains 9600 nucleotide bases long and is covered by an icosahedral nucleocapsid which is further surrounded by a lipid bilayer and glycoproteins. HCV is grouped into 6 major genotypes that exhibit at least 30% variation in nucleotide sequence from one another. This genetic variation within the population is a powerful selection mechanism for resistance to both medicinal drugs and evasion of the immune system.

Genotyping and assessment of viral load in HCV patients is important for planning the therapeutic strategies. In the recent past, the conventional antiviral therapy against HCV was either monotherapy with interferon (IFN) that may be pegylated or in combination with ribavirin. The penultimate response to therapy and its duration was genotype dependent. The viral...
load is monitored at different time intervals during therapy. While up to 80% of the genotypes 2 and 3 show good response to treatment with pegylated or standard IFN-alfa and ribavirin, genotypes 1, 4, 5 and 6 show poorer response. With the development of direct-acting antivirals (DAAs) comprising at least three other classes of drugs: NS5A inhibitors such as ledipasvir and daclatasvir; NS5BRNA dependent RNA polymerase inhibitors comprising nucleoside/nucleotide analogues (NPIs) such as sofosbuvir and non-NPIs such as dasabuvir, IFN-free treatment regimens have been made possible. With the use of these second-generation DAAs, SVR rates of over 90% have reported. Most recently, DAA have become the standard of treatment of Hepatitis C infection. Duration of treatment in uncomplicated cases of HCV-related chronic liver disease is 12 weeks.

The worldwide prevalence of Genotype 3 (54.3 million cases [30.1% of the total]) is considered second most after Genotype 1, with the highest seroprevalence in southern Asia. The HCV genotype analysis has not only effect on disease presentation but is also valuable for antiviral therapy, counselling and the proper management. It has been stated there has been a decrease in the recognition of HCV related liver disease due to lower Amino-transferase activity in patients with chronic renal disease as compared to nonuremic patients. Hence more aggressive approach for diagnosis is required.

Very few studies have been done in this part of country. So this study has been undertaken with the following aim and objectives.

Aims and Objectives

To study prevalence of Hepatitis C Infection (HCV) by Real Time Polymerase Chain Reaction and to identify genotype of Hepatitis C Virus (HCV) in Chronic Renal Disease patients undergoing hemodialysis in tertiary care hospital.

MATERIALS AND METHODS

The study was conducted in a tertiary care hospital for a period of two years. The sample taken was plasma separated from blood. 5 ml blood was collected in vial by venipuncture in two vacutainer tubes containing dipotassium ethylene diamine tetra acetate (EDTA). The tube was centrifuged at 2500 rpm for 10 minutes and the supernatant plasma was stored at -20°C for further processing, i.e. HCV-RNA viral load and HCV genotyping (in samples positive for HCV-RNA viral load).

240 patients were recruited for the study. Real time PCR for quantitative detection of HCV-RNA was performed in all patients. HCV-RNA was quantified using standard RNA extraction and real time amplification kits using Taqman principle along with quantitation standards. Real Time PCR for HCV genotyping was performed in HCV-RNA positive patients.

Inclusion Criteria

Patients of Chronic Renal Disease undergoing hemodialysis were recruited for study.

Exclusion Criteria

All other confirmed patients of hepatitis, including alcohol and drug induced hepatitis (anti tubercular drugs, halothane) were excluded.

Statistical Analysis

Data was collected using a structured proforma. Data entry was done in Microsoft Excel spreadsheets. Data analysis was done using Statistical Package for Social Sciences (SPSS Ver. 18.0). Percentages were calculated for all categorical variables. Mean and median were calculated for all continuous variables such as age, and HCV-RNA viral load. P value was calculated for categorical outcomes. A p-value < 0.05 was considered as statistically significant.

RESULTS

A total of 240 Chronic Renal Disease patients (161 male and 79 female patients) who underwent haemodialysis were tested for HCV RNA out of which 128 (53.33%) were positive and 112 (46.67%) were negative (Fig.1).

Prevalence of HCV-RNA Genotype

The detection of HCV-RNA genotype was carried out in 128 patients who were positive for HCV-RNA viral load by real time polymerase chain reaction.

Out of these 128 patients, 73 (57.04%) patients were positive for HCV-RNA genotype and 55 (42.96%) were found to be negative for HCV-RNA genotype due to low viral load (Table 1).

<table>
<thead>
<tr>
<th>Total number of samples for Genotype</th>
<th>Positive</th>
<th>Negative (Low RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>73 (57.04%)</td>
<td>55 (42.96%)</td>
</tr>
</tbody>
</table>

Distribution pattern of HCV-RNA Genotype

In our study, HCV-RNA genotype was positive in 73 patients. The genotypes detected were Genotype 1, 2, 3 and 4. As per our kit manufacturer’s manual, Genotype 5 and 6 could not be detected. Distribution pattern of HCV-RNA Genotype is shown in Fig.2.
1. Out of 73 samples positive for genotype.
2. Genotype 1 is 37 (50.68%)
3. Genotype 2 is 7 (9.59%)
4. Genotype 3 is 25 (34.25%)
5. Genotype 4 is 4 (5.48%)

Thus it was observed in our study genotype 1 is most prevalent genotype observed in 37 (50.68%) patients in our study followed by genotype 3 with 25 (34.25%) patients, genotype 2 with 7 (9.59%) patients and genotype 4 with 4 (5.48%) patients.

**Quantitative distribution of HCV-RNA viral load in different genotypes**

In positive HCV RNA viral load out of 73 positive HCV-RNA genotype patients, in category of HCV-RNA viral load with 1000-5000 IU/ml maximum prevalence was observed for genotype 1 (53.33%) followed by genotype 3 (26.67%) and genotype 2 (20%). Among viral load of 5000-1000000 IU/ml the distribution was maximum for genotype 1 (45.71%) followed by genotype 2 (40%), genotype 2 (11.43%). In very high quantitative HCV viral load genotype 1 was again most prevalent (56.52%) followed by genotype 3 (30.43%) and genotype 4 (5.48%). There was no statistical significance observed (P value = 0.190) in quantitative distribution of HCV-RNA viral load in different genotypes.

**DISCUSSION**

A total of 240 blood samples (no repeat sample) from patients of Chronic Renal Disease undergoing hemodialysis were recruited for study. All other confirmed patients of hepatitis, including alcohol and drug induced hepatitis (anti tubercular drugs, halothane) were not included in the study.

A total of 240 patients were evaluated in the study for HCV-RNA quantification (viral load) by real time polymerase chain reaction. Out of these patients, 128 (53.33%) were positive and 112 (46.67%) of them were found to be negative for HCV-RNA viral load. Therefore the prevalence of HCV-RNA in our study was found to be 53.33% (Fig 1). This is found similar to studies in spain (10 to 50%)22 and in many countries of northern Africa, Asia and South America (ranges between 10%-70%),23. The worldwide prevalence of HCV infection among HD patients varies widely, with estimates ranging from 5% to approximately 60% depending on geographic location.24-25.

Out of 240 patients, HCV-RNA genotype detection was carried out in those patients who were positive for HCV-RNA viral load by real time polymerase chain reaction. Total number of patients positive for HCV-RNA viral load were 128, therefore HCV-RNA genotype was conducted in these 128 patients.

Out of these 128 patients, 73 (57.04%) patients were positive for HCV-RNA genotype and 55 (42.96%) were found to be negative for HCV-RNA genotype due to low viral load. There were 56 (76.71%) males and 17 (23.29%) females.

The genotypes detected were Genotype 1, 2, 3 and 4 as our reagent kit was compatible for the same. Genotype 5 and 6 could not be detected. The distribution of genotypes was found to be: genotype 1 accounted for 37 (50.68%), genotype 2 for 7 (9.59%), genotype 3 for 25 (34.25%) and genotype 4 for 4 (5.48%).

**Fig 2** Distribution pattern of HCV-RNA Genotype (n=73)

**Gender distribution of HCV-RNA genotype**

Out of 73 patients positive for HCV-RNA genotype, 56 (76.71%) were males and 17 (23.28%) were females. It is shown in Table 2.

**Table 2** Gender distribution of HCV-RNA genotypes (n=73) (Figures in parenthesis show percentages)

<table>
<thead>
<tr>
<th>Total number of HCV-RNA viral load positive samples</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td>56 (76.71%)</td>
<td>17 (23.29%)</td>
</tr>
</tbody>
</table>

Genotype distribution among different age groups

The total number of patients who were positive for HCV-RNA genotype was 73 out of 128 patients positive for HCV-RNA viral load.

**These 73 patients were categorized in three age groups**

1. <41 years
2. 41-60 years
3. >60 years

Prevalence of different genotypes in different age groups is shown in Table 3 and Fig 43 in which it was observed that the distribution of age group <41 yrs was maximum for genotype 1 (66.67%) followed by genotype 2 (33.33%). For age group of 41-60 yrs the most prevalent was genotype 1 (53.97%), genotype 2 (7.94%), genotype 3 (31.75%) and genotype 4 (6.35%). In age group of >60 yrs genotype 3 was most prevalent genotype (34.25%) followed by genotype 1 (14.29%) and genotype 2 (14.29%). There was no statistical significance of age distribution (P value=0.184) in 73 patients with positive HCV-RNA genotype.
Therefore, the most prevalent genotype in the study was found to be genotype 1. It is followed by genotype 3, 2 and genotype 4. This finding is very different from other studies on genotype conducted in India as most of the studies mention the prevalence of genotype 3 particularly in North India. Genotype 3 is most common in the Indian subcontinent while genotype 4 is the most common genotype seen in Africa and the Middle East. Genotype 5 can be found in South Africa and as mentioned above, genotype 6 can be found in south-east Asia26. A decade-long experience from a tertiary care hospital in South India27 over a study period of ten years from 2002 onwards showed that consistently, genotype 3 (63.85%) is more prevalent followed by genotype 1, 4 and 6 (25.72%, 7.5% and 2.7%). Our results differ from these studies and may be due to change in sociogeological patterns of the migratory population.

In a study done at Ege University Hospital in Turkey analysis of the genotype distribution according to age showed that patients infected with type 1 (mean ± SD age, 53.6 ± 12.4 years) were significantly older than patients infected with non-1 genotypes (mean ± SD age, 42.2 ± 16.8 years; p = 0.03). When genotype distribution (genotype 1 and non-1 (genotypes 2, 3, and 4) were investigated in relation to gender, no significant difference was found (Chi-square = 0.333, p > 0.05)28. In a study done in Italy no statistical difference was observed in the gender distribution considering the overall genotypes 1 and 2 (61.6 vs. 65.5% for genotype 1 and 27.7 vs. 31.1% for genotype 2), even though subtype 1b was predominant among females (56.4 vs. 44.7%, p < 0.05). Conversely, genotype 3 was more frequent in males than in females (9.7 vs. 2.9%, p < 0.005), possibly due to a higher prevalence of intravenous drug addiction among men7. In our study the results showed that genotype 1 was found in older age groups. It was also seen that genotype 1 and genotype 3 were observed to be predominant among males. There was no statistical significance in age and gender distribution (P value 0.184 and 0.623 respectively).

In a study done in a tertiary care hospital in New Delhi, Genotype 1 was associated with a significantly (P<0.001) higher viral load as compared to genotypes 3 and 226. In a study done in Lahore Genotype 3 is associated with the lowest viral loads, and infection by genotype 1a is associated with higher levels of the serum biomarkers31. The similar result was observed in our study as genotype 1 was found to be more prevalent in higher viral load as compared to genotype 3 in lower viral load. However it fell short of statistical significance (Table 3).

CONCLUSION

The prevalence of HCV infection is much higher in hemodialysis patients (CKD Stage 5D) than in the general population and is associated with an increased mortality rate. The distribution pattern of HCV-RNA genotype in our study showed a prevalence of genotype 1 followed by 3, 2 and 4. Our results differ which may be due to change in sociogeological patterns of the migratory population.

Knowledge of regional distribution of HCV genotypes is important since this could influence configuration of diagnostic assays as well as vaccine designs. Any change in distribution of HCV genotypes needs to be closely monitored in further studies as this could have therapeutic implications.

The identification and characterization of HCV genotypes have implications for HCV treatment and vaccine development. Also, it is clear that HCV genotypes are important epidemiologic markers as geographical distribution of HCV genotypes may be useful for epidemiological purposes. HCV genotyping in combination with HCV-RNA may be beneficial in the management of chronic hepatitis C and in the selection of candidates for treatment modalities and to decide the period of treatment.

Therefore in this scenario, the gold standard for diagnosis of HCV infection should be considered as detection of HCV-RNA in serum by polymerase chain reaction (PCR) assay.

References


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